

ResearchOnline@JCU

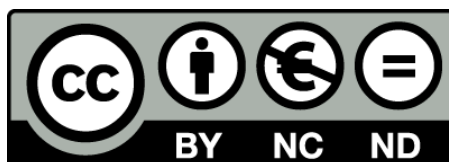
This is the **Accepted Version** of a paper published in the
Journal: Brain, Behaviour and Immunity

Mifsud, Karen R., Gutirrez-Mecinas, María, Trollope, Alexandra F., Collins, Andrew, Saunderson, Emily A., and Reul, Johannes M.H.M. (2011) *Epigenetic mechanisms in stress and adaptation*. Brain, Behavior, and Immunity, 25 (7). pp. 1305-1315.

<http://dx.doi.org/10.1016/j.bbi.2011.06.005>

© 2015. This manuscript version is made available under
the CC-BY-NC-ND 4.0 license

<http://creativecommons.org/licenses/by-nc-nd/4.0/>



Manuscript Number: BBI-D-11-00104R1

Title: Epigenetic mechanisms in stress and adaptation

Article Type: Named Series: Epigenetics

Section/Category: Neurosciences

Keywords: Stress; adaptation; epigenetic; learning; memory; DNA methylation; histone modification; ERK; MAPK; NMDA; glucocorticoid; c-Fos; Egr-1; immune; inflammation; cytokine; hippocampus; dentate gyrus; HPA axis

Corresponding Author: Professor Johannes M.H.M. Reul,

Corresponding Author's Institution: University of Bristol

First Author: Karen R Mifsud

Order of Authors: Karen R Mifsud; Maria Gutierrez-Mecinas; Alexandra F Trollope; Andrew Collins; Emily A Saunderson; Johannes M.H.M. Reul

Manuscript Region of Origin: UNITED KINGDOM

Abstract: Epigenetic mechanisms are processes at the level of the chromatin that control the expression of genes but their role in neuro-immuno-endocrine communication is poorly understood. This review focuses on epigenetic modifications induced by a range of stressors, both physical and psychological, and examines how these variations can affect the biological activity of cells. It is clear that epigenetic modifications are critical in explaining how environmental factors, which have no effect on the DNA sequence, can have such profound, long-lasting influences on both physiology and behavior. A signaling pathway involving activation of MEK-ERK1/2, MSK1, and Elk-1 signaling molecules has been identified in the hippocampus which results in the phospho-acetylation of histone H3 and modification of gene expression including up-regulation of immediate early genes such as c-Fos. This pathway can be induced by a range of challenging experiences including forced swimming, Morris water maze learning, fear conditioning and exposure to the radial maze. Glucocorticoid (GC) hormones, released as part of the stress response and acting via glucocorticoid receptors (GRs), enhance signaling through the ERK1/2/MSK1-Elk-1 pathway and thereby increase the impact on epigenetic and gene expression mechanisms. The role of synergetic interactions between these pathways in adaptive responses to stress and learning and memory paradigms is discussed, in addition we speculate on their potential role in immune function.

Review article for Named Series Epigenetics in *Brain, Behavior and Immunity*

BBI-D-11-00104 Revised

Epigenetic mechanisms in stress and adaptation

Karen R. Mifsud, María Gutiérrez-Mecinas, Alexandra F. Trollope, Andrew Collins, Emily A. Saunderson and Johannes M.H.M. Reul*

Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, University of Bristol, Dorothy Hodgkin Building, Whitson Street, Bristol, BS1 3NY, United Kingdom

*Corresponding author.

Professor Johannes M.H.M. Reul

Tel: +44 117 331 3137

E-mail: Hans.Reul@bristol.ac.uk

The authors declare that there are no conflicts of interest.

Word count: (excluding references, tables and figures): 7067

Figures: 2

Tables: 1

References: 112

1
2 **Review: Epigenetic mechanisms in stress and adaptation**
3
4
5
6

7 **Abstract**
8

9
10 Epigenetic mechanisms are processes at the level of the chromatin that control the expression
11 of genes but their role in neuro-immuno-endocrine communication is poorly understood. This
12 review focuses on epigenetic modifications induced by a range of stressors, both physical and
13 psychological, and examines how these variations can affect the biological activity of cells. It
14 is clear that epigenetic modifications are critical in explaining how environmental factors,
15 which have no effect on the DNA sequence, can have such profound, long-lasting influences
16 on both physiology and behavior. A signaling pathway involving activation of MEK-
17 ERK1/2, MSK1, and Elk-1 signaling molecules has been identified in the hippocampus
18 which results in the phospho-acetylation of histone H3 and modification of gene expression
19 including up-regulation of immediate early genes such as c-Fos. This pathway can be induced
20 by a range of challenging experiences including forced swimming, Morris water maze
21 learning, fear conditioning and exposure to the radial maze. Glucocorticoid (GC) hormones,
22 released as part of the stress response and acting via glucocorticoid receptors (GRs), enhance
23 signaling through the ERK1/2/MSK1-Elk-1 pathway and thereby increase the impact on
24 epigenetic and gene expression mechanisms. The role of synergetic interactions between
25 these pathways in adaptive responses to stress and learning and memory paradigms is
26 discussed, in addition we speculate on their potential role in immune function.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 **Introduction to stress**
2
3

4 The nervous, endocrine and immune systems engage in intense communication throughout
5 the mammalian physiological system via neuronal, hormonal and immunological networks
6 respectively. These systems are widely integrated with each other and allow the organism to
7 adapt to environmental changes and challenges. Stress has been defined as ‘a threat, real or
8 implied, to the maintenance of a narrow range of vital homeostatic parameters necessary for
9 survival’ (McEwen, 2000). Stress induces a number of biological responses which enables
10 the organism to adapt to the challenge and increase its likelihood of survival. Immediate
11 activation of the sympathetic nervous system releases adrenaline from stores in the adrenal
12 medulla, preparing the organism for a fight or flight response. In addition, stress increases
13 levels of neurotransmitters such as serotonin (5-HT) and noradrenaline (NA) in specific brain
14 regions (i.e. hypothalamus, hippocampus) (Linthorst et al., 1995a; 1996; Linthorst et al.,
15 2002). 5-HT and NA act at the hypothalamus to activate the hypothalamic-pituitary-adrenal
16 (HPA) axis resulting in the release of GCs from the adrenal cortex into the circulation
17 (Carrasco and Van de Kar, 2003). It is hypothesised that the rise in extracellular 5-HT in the
18 hippocampus supports the animal’s risk assessment of the nature of the stressor so it can
19 make appropriate adaptations (Linthorst and Reul, 2008).
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **Animal models for investigating the effects of acute stress**
46

47 Researchers investigating the effects of stress *in vivo* have developed a number of behavioral
48 tests designed to model neuropsychiatric disorders, for review see Nestler and Hyman (2010).
49
50 In many stress studies animals are exposed to stressors for a limited amount of time and
51 subsequent changes in physiology and behavior determined. Stressors are generally described
52 as either physical or psychological but in reality most stressors present both types of stress
53 and therefore their ‘classification’ is based on which biological system (physical or
54
55
56
57
58
59
60
61
62
63
64
65

1 psychological) is affected the most. Physical stressors, such as a hot or cold environment,
2 have a direct effect on the body, disturbing homeostatic processes and influencing the
3 physiological state of an organism. In contrast, psychological stressors involve higher brain
4 areas, such as the neocortex, amygdala and hippocampus, to process and/or interpret
5 information about the threat. Consequently, in case of psychological stress, the threat may be
6 implied but not necessarily experienced (Herman, 2003).
7
8
9
10
11
12
13
14
15
16

17 Both physical and psychological stress activate the HPA axis leading to downstream
18 adrenocorticotrophic hormone (ACTH) release from the anterior pituitary and subsequent
19 release of GCs (cortisol in humans, corticosterone in rodents) from the adrenal cortex into the
20 circulation. GCs were so named after their effects on glucose metabolism were discovered.
21 Circulating GCs, however, have equally important roles in modulating the immune response
22 and in cognitive processes such as memory formation (see next section). Despite both
23 physical and psychological stressors activating the same central HPA pathway, a number of
24 studies have provided evidence that the body responds to the different types of stress in
25 distinct ways (Dayas et al., 2001; Yuen et al., 2009). Furthermore, because of its nature,
26 psychologically stressful events lead to profound, long-term changes in behavior.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

Role of memory formation in the stress response

43 Memory formation is the storage of information which can be recalled in the future. The
44 processes involved in the formation of memories have been the focus of numerous studies
45 spanning over 30 years but a definitive pathway is still being elucidated. Biochemical
46 pathways leading to memory formation can be examined through the use of behavioral tests
47 such as the Morris water maze, Pavlovian fear conditioning, radial maze and others - a
48 summary of the most common behavioral tests for stress/learning and memory studies are
49 outlined in Table 1. All of these tests have a 'stressful' component which must be taken into
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 account when interpreting experimental findings. Indeed in recent years there has been
2 considerable overlap between investigations into stress responses and learning and memory
3 processes, with signaling pathways emerging that are common to both areas. Long term
4 responses to stress include the formation of episodic memories which increase the chance of
5 avoiding the stressful challenge in the future or improve the ability of the organism to cope
6 with any subsequent repeat exposure to the stressor (Reul and Chandramohan, 2007; Reul et
7 al., 2009) This is, in part, due to the strong consolidation of memories by the induction of
8 stress hormones such as GCs, however, the exact mechanism for this consolidation remains
9 unclear (Oitzl and de Kloet, 1992; Cordero and Sandi, 1998; De Quervain et al., 2009; Sandi,
10 2011).

26 **Epigenetics**

27 As discussed in the first review of this series (Mathews and Janusek, 2011), epigenetic
28 modifications change the expression of genes without altering the DNA sequence. These
29 modifications can occur on the DNA itself to influence gene expression by changing the
30 accessibility of genes to transcription/translation factors. Alternatively, other distinct
31 epigenetic modifications can occur on the highly conserved tails of histone proteins,
32 responsible for organizing DNA into chromatin, thereby affecting chromatin assembly and
33 organization of DNA.

34 According to the National Institute of Health, epigenetic changes can be ‘heritable changes in
35 gene activity and expression (in the progeny of cells or of individuals) and also stable, long-
36 term alterations in the transcriptional potential of a cell that are not necessarily heritable’. The
37 latter aspect explains how environmental influences and life experiences can result in
38 differences in gene expression and downstream biological changes, even after the initial

1 influence has disappeared. The different types of epigenetic modifications influencing gene
2 expression are reviewed extensively by Kouzarides (2007) and Mathews and Janusek (2010).
3
4 This review focuses on DNA methylation, histone methylation, phosphorylation and
5 acetylation and as such, only these epigenetic modifications will be discussed.
6
7
8
9

10 11 *DNA modifications*

12 DNA methylation occurs when a methyl group is added to one of the pyrimidine bases of
13 DNA. Methylation occurs on the 5th carbon of cytosine residues (5mC) situated adjacent to a
14 guanine residue (CpG site). Parts of DNA sequences with a high concentration of CpG
15 residues are referred to as CpG ‘islands’ and generally located at the start of the gene
16 sequence within the promoter region. The methylation of DNA within the promoter region of
17 a gene may be why this modification has such a profound effect on gene expression,
18 generally silencing the expression of the respective gene (Illingworth and Bird, 2009).
19
20
21
22
23
24
25
26
27
28
29
30
31

32 DNA methylation can occur by two type of enzymes; methyl-transferases *de novo*, which set-
33 up methylation marks during early development and are critical for the survival of organisms,
34 and maintenance methyl-transferases which are essential for maintaining the methylation
35 code (Wu and Zhang, 2010). The importance of demethylation has been investigated to a
36 greater extent in recent years as it has become apparent that DNA methylation status can
37 change quite rapidly (Wu and Zhang, 2010). The exact biochemical processes involved in
38 DNA demethylation remains unclear but there are a number of mechanisms currently being
39 discussed including enzymatic removal, deamination and base excision repair, nucleotide
40 excision repair, oxidative demethylation and radical S-adenosylmethionine (SAM)-based
41 demethylation (for review see Wu and Zhang (2010)).
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

In addition to methylation and demethylation, mammalian DNA can be hydroxylated (oxidation of CH group to form COH) at the carbon 5 position of methylated cytosine residues (Penn et al., 1972) but the induction and function of this hydroxy-methylation (5hmC) modification is unknown. More recent studies (Tahiliani et al., 2009; Zhang et al., 2010) have identified 5-methylcytosine (5mC) hydroxylases like ten-eleven translocation proteins (TET1, TET2 and TET3) as enzymes responsible for hydroxylation of methylcytosine but the function of this mark still remains unclear. It has been proposed that 5hmC facilitates passive demethylation (promoting gene transcription) by preventing DNMT's maintaining the methylation status of DNA, however, this is yet to be confirmed (Tahiliani et al., 2009). Hydroxy-methylation of DNA recruits 5hmC-specific factors and prevent the association of some 5mC-specific enzymes/transcription factors in DNA methylation assays and cancer cell lines, indicating distinct roles for the two types of cytosine modification in gene expression (Valinluck and Sowers, 2007; Tahiliani et al., 2009; Ko et al., 2010).

Histone modifications

Histone proteins are responsible for organizing DNA into chromatin which can exist in a densely-packed 'closed' configuration or in a loose, 'open' state, which is required for gene transcription. There are four core histone proteins, H2A, H2B, H3 and H4, each with a highly conserved N-terminal tail which can be modified at certain sites by a range of specific enzymes to influence the structure and function of the chromatin.

Histone methylation occurs on either arginine or lysine residues in histones H3 and H4. The enzymes which add (methyl-transferases) or remove (demethylases) methyl groups are specific to the residue on which they act, either lysine or arginine, and can also be specific for

1 the location or site of the residue within the histone tails. For example, mixed lineage
2 leukemia proteins (MLLs) are methyl-transferases which act almost exclusively to methylate
3
4 lysine residue 4 of histone H3 (Cosgrove and Patel, 2010). The function of the methylation
5
6 mark depends on the site on which it occurs; lysine methylation of H3K4 (lysine 4 of histone
7
8 3) is associated with transcriptionally active chromatin and gene transcription whereas
9
10 methylation on H3K9 is associated with gene silencing (Kouzarides, 2007; Akbarian and
11
12 Huang, 2009). The consequence of methylation is also dependant on how many methyl
13
14 groups are added to each residue; lysine residues hold one, two or three methyl groups and
15
16 are classified as mono, di or trimethylated respectively, whereas arginine residues can only
17
18 hold up to two methyl groups (Kouzarides, 2007). The function of methylated H3K20 varies
19
20 greatly with methylation status, mono-, di- and trimethylation of this site has been associated
21
22 with gene transcription, gene repair and gene silencing respectively (Balakrishnan and
23
24 Milavetz, 2010). The consequence of epigenetic marks is further complicated by the
25
26 interaction of different modifications on each other; for example, protein arginine methyl-
27
28 transferase (PRMT) methylation of H3R2 prevents methylation of H3K4 by MLL and
29
30 therefore inhibits subsequent gene expression *in vitro* (Hyllus et al., 2007).
31
32
33
34
35
36
37
38
39
40

41 Histone phosphorylation occurs when a phosphate group is added to an amino acid residue
42
43 (usually serine) in the highly conserved histone tail by specific kinases and can be removed
44
45 by specific phosphatases (dephosphorylation) (Thomson et al., 1999b; 1999a; Clayton et al.,
46
47 2000; Soloaga et al., 2003). The phosphorylation status of chromatin is determined by the net
48
49 activity of kinases and phosphatases, influenced by signaling cascades. Phosphorylation of
50
51 certain residues is often associated with other epigenetic marks, for example if histone H3 in
52
53 dentate gyrus neurons is phosphorylated at serine 10 then it is also generally acetylated at
54
55 lysine 14 (Chandramohan et al., 2007). This co-modification is associated with the opening of
56
57
58
59
60
61
62
63
64
65

1
2
3 previously condensed chromatin (Cheung et al., 2000; Clayton et al., 2000; Nowak and
4
5
6 Corces, 2000) .

7
8 Histone acetylation occurs when an acetyl group (COCH₃) is added to a lysine residue by
9
10 histone acetyl-transferases (HATs). Once again these enzymes act at distinct residues/sites
11
12 within the N-terminal tail of histone proteins. Given the neutralization of the negative lysine
13
14 charge by the addition of a positively charged acetyl group, histone acetylation is the
15
16 modification most likely to decondense chromatin and expose previously silent genes for
17
18 transcription (Kouzarides, 2007). This epigenetic mark is therefore almost exclusively
19
20 associated with active gene transcription for numerous genes studied. Acetyl groups are
21
22 removed by histone deacetylases (HDACs).
23
24
25
26
27
28

29
30 Some epigenetic marks such as methylation of DNA can be passed on to subsequent
31
32 generations through mitotic and meiotic cell division (Nakayama et al., 2000; Schreiber and
33
34 Bernstein, 2002; Champagne, 2008). This is an interesting property which is not shared by
35
36 alternative mechanisms of altering gene expression (i.e. the influence of transcription
37
38 factors). Although changes in the epigenetic profile of DNA were traditionally thought to be
39
40 hereditary, recent studies indicate these changes can be transient (Ng et al., 2009). Epigenetic
41
42 modifications are therefore a set of biochemical tools which control the expression of genes
43
44 and are themselves controlled by signaling mechanisms and provide another level at which
45
46 the environment can impact on gene expression (Figure 1).
47
48
49
50
51
52
53

54 **Role of epigenetic mechanisms in the long-term impact of early life stress**

55
56 The epigenetic profile of rats is profoundly influenced by the maternal behavior they
57
58 experienced during the first week of life (Weaver et al., 2004). Rats which received high
59
60
61
62
63
64
65

1 levels of maternal care (HMC rats, maternal care defined as licking, grooming and arched-
2 back nursing) during the first week of life were less anxious, less sensitive to stress in adult
3 life and had higher levels of GR expression in the hippocampus compared with rats
4 experiencing low levels of maternal care as pups (LMC rats).
5
6
7
8
9

10
11 Analysis of the methylation state of the GR promoter during the development of HMC or
12 LMC pups revealed changes in the methylation status of a specific cytosine residue (site 16
13 on exon 1₇), which corresponded to a specific region in the GR promoter containing a
14 consensus sequence for a transcription factor known as early growth response factor 1 (Egr-
15 1). At the embryonic stage site 16/exon 1₇ is unmethylated but DNA methylation of this site
16 occurs shortly after birth. In HMC pups demethylation of site 16/exon 1₇ occurs during the
17 first week of life, whereas this site in LMC rats remains methylated. Since this site contains a
18 consensus sequence for Egr-1, the association of Egr-1 protein with the GR promoter was
19 analysed by chromatin immuno-precipitation assay (ChIP) and found to be 3-fold higher in
20 HMC rats compared with LMC rats. ChIP analysis of GR also found higher levels of
21 acetylation at histone H3 lysine 9 (H3K9), a marker for uncondensed, transcriptionally active
22 chromatin (Weaver et al., 2004).
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

44 Prolonging histone acetylation by intracerebroventricular (i.c.v) administration of histone
45 deacetylase (HDAC) inhibitor trichostatin A (TSA) in LMC rats supported a role for histone
46 acetylation in the response by promoting a phenotype closely associated with that of HMC
47 rats (Weaver et al., 2004). Likewise, when Egr-1 binding to the GR promoter was inhibited
48 by DNA methylation at site 16/exon 1₇ the HMC phenotype (less anxious, less sensitive to
49 stress and higher GR expression in hippocampus) disappeared indicating a key role for
50 demethylation of DNA in this phenotype (Weaver et al., 2004). Later studies found that the
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 level of maternal care experienced when pups by female rats determined what kind of mother
2 they would themselves become as a consequence of epigenomic changes in a different gene,
3
4 namely the estrogen receptor gene located in the medial preoptic area, highlighting the trans-
5 generational effects of epigenetic modifications (Champagne, 2008). Generally it appears that
6
7 HMC rats become HMC mothers and LMC rats become LMC mothers (Champagne, 2008).
8
9

10
11
12
13
14 A link has also been demonstrated between early life experiences and adult behavior in
15 humans. Numerous clinical studies have shown a positive association between childhood
16 trauma and increased risk of developing psychopathological disorders such as depression as
17 an adult (Heim et al., 2008). Children experiencing abuse, neglect or other adverse
18 experiences displayed a sensitized stress response, GC resistance, increased corticotropin-
19 releasing factor (CRF) activity, immune activation and reduced hippocampal volume; all
20 features which have been observed in some depressed patients as adults (Heim et al., 2008).
21
22 Reduced GR mRNA expression in the hippocampus of suicide victims who had been abused
23 as children was associated with epigenetic changes in the neuron-specific GR promoter
24 (NR3C1) when compared with suicide victims with no history of childhood abuse (McGowan
25 et al., 2009). There were also higher levels of DNA methylation in the hippocampal GR
26 promoter in abuse victims compared with non-abuse controls; this pattern of methylation
27 resulted in reduced binding of transcription factor Egr1 and decreased levels of Egr1-induced
28 gene transcription when investigated *in vitro* (McGowan et al., 2009). Based on these studies,
29 epigenetic modifications play a key role in mediating the effect of early life experiences on
30 responses to stress in adulthood.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Epigenetics: stress studies and signaling pathways

1
2 Our recent studies have investigated the effect of acute stressors on epigenetic modifications
3
4 in brains of adult rats. The primary stressor used in our studies is the forced swim test and we
5
6 are particularly interested in the subsequent immobility response displayed by the rats in the
7
8 forced swim retest (24 h after initial test). There is controversy surrounding the interpretation
9
10 of the animal's immobility response. Originally the immobility response was viewed as a
11
12 'depressive' behavior because it was thought that the animal had given up and showed
13
14 despair (Porsolt et al., 1977; Lucki, 1997). In addition, the effects of some antidepressant
15
16 drugs to lower the immobility response seemed to support this notion. On the other hand,
17
18 acute administration of many antidepressants evokes arousal through central release of
19
20 serotonin, noradrenaline and/dopamine which may provide an alternative explanation for the
21
22 reduced immobility behavior. Furthermore, the effect of three administrations of
23
24 antidepressant drug in the Porsolt forced swim test design is hard to reconcile with the
25
26 clinical effects of antidepressants which take at least 3 weeks of treatment to emerge. Our
27
28 group and others feel that the immobility response is an adaptive response to the knowledge
29
30 that they cannot escape making it more appropriate to conserve energy to maximize the
31
32 chance of survival (De Pablo et al., 1989; West, 1990; Korte, 2001; Bilang-Bleuel et al.,
33
34 2005; Nestler and Hyman, 2010). The rapid adoption of immobility behavior in the retest
35
36 supports the idea that the subject remembers the initial test and conserves its energy by
37
38 floating. Further support of immobility being an adaptive response comes from forcing the
39
40 rats to swim in water at different temperatures. Normally the rats are swum at 25⁰C, however,
41
42 when rats are swam at 19⁰C rats they show increased struggling and reduced immobility
43
44 during the retest, possibly because of the extreme loss of body temperature (~12⁰C). In
45
46 contrast, rats that swam at 35⁰C showed high levels of immobility and very little struggling
47
48 (Linthorst et al., 2008). This experiment underscores the immobility behavior being an
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

adaptive response to the combined physical and psychological stress. The observed ability of the rats to recall the initial experience, as shown by the increase in immobility in the retest, lasts at least 4 weeks from initial test (Gutiérrez-Mechinas, Collins, & Reul; unpublished observations). Once again this finding supports a role of learning and memory processes in the adaptive immobility behavior shown and refutes the depression hypothesis.

Immunofluorescent staining confirmed epigenetic modifications were taking place in distinct brain areas with positive staining for phosphorylation of serine 10 and acetylation of lysine 14 in histone H3. Under baseline conditions very low levels of H3 phospho-acetylation were found in the dentate gyrus, neocortex, amygdala and striatum (Bilang-Bleuel et al., 2005; Chandramohan et al., 2007). On exposure to forced swim stress there was a marked increase in phospho-acetylation of histone H3, specifically in the dentate gyrus, which did not occur after exposure to purely physical stresses such as cold environment and ether exposure. This finding indicates that the psychological component of the stress may be responsible for the observed increase in H3 phospho-acetylation (Bilang-Bleuel et al., 2005).

N-methyl-D-aspartate (NMDA) receptor blockade, using receptor antagonist MK-801, prevented the forced swim induced increase in H3S10p-K14ac positive neurons in the dentate gyrus of rats and also reduced the associated immobility behavior in the 1-day retest (Chandramohan et al., 2008). Neurons in the dentate gyrus are under tonic inhibitory control by gamma amino-butyric acid (GABA)-ergic interneurons. FG-7142 (β -carboline-3-carboxylic acid N-methylamide), a GABA-A receptor partial inverse agonist attenuated the GABAergic control of granule neurons in the dentate gyrus, resulting in a rise in the number of H3S10p-K14ac-positive neurons in this hippocampal structure (Papadopoulos et al., 2010). The proportion of granule neurons in the dentate gyrus that overcome the inhibitory

1 regulation is dependent on the strength of the stimulus. Most psychological stressors
2 (predator exposure, novel environment etc.) activate <5% of dentate gyrus granular neurons
3 from inhibitory control. In contrast, much stronger stimuli such as electroconvulsive shock or
4 injection of depolarizing drugs can overcome virtually all inhibitory GABAergic control
5 resulting in activation of all dentate granule neurons (Green and Vincent, 1987; Crosio et al.,
6 2003).

7
8
9
10
11
12
13
14
15
16
17 Once the inhibitory control is overcome by excitatory inputs (mainly glutamatergic),
18 glutamate binds to NMDA receptors resulting in a rise in intracellular calcium (Ca^{2+})
19 concentration. Increased intracellular Ca^{2+} levels trigger a number of biochemical signaling
20 cascades including the activation of adenylate cyclase (and subsequent protein kinase A
21 (PKA) activation), Ca^{2+} -calmodulin kinase II (CAMKII) and the mitogen-activated protein
22 kinase (MAPK) cascade leading to activation of the extracellular-regulated protein kinases 1
23 and 2 (ERK1/2). Inhibition of ERK1/2 activation, using an inhibitor (i.e. SL-327) of the
24 upstream MAPK ERK kinase (MEK), abolished phospho-acetylation of histone H3 and the
25 subsequent immobility response in response to forced swim stress (Chandramohan et al.,
26 2008). ERK1/2 targets a number of downstream kinases including mitogen and stress-
27 activated kinases 1 and 2 (MSK1/2). MSK1/2 double knock-out mice subjected to the same
28 forced swim protocol (although initial test was for 10 min as opposed to 15 min) showed no
29 increase in phospho-acetylation of histone H3 and the immobility response was also blocked
30 compared with control mice (Chandramohan et al., 2008). This study shows that blocking
31 ERK1/2 and MSK signaling prevents forced swim induced epigenetic changes and
32 subsequent immobility behavior during the retest, indicating a key role for these signaling
33 molecules in the adaptive behavioral response to stress.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Once activated, MSK can phosphorylate residues on histone tails including S10 which may explain its critical role in inducing epigenetic modifications (Figure 2), however, MSK has no known acetylase activity (Hauge and Frödin, 2006; Arthur, 2008; Drobic et al., 2010). MSK can also phosphorylate cAMP responsive element (CRE) binding protein (CREB) which after dimerization can bind to CRE sites in the promoters of many cAMP- and Ca²⁺-responsive genes thereby stimulating gene expression (Figure 2). Furthermore, CREB can recruit a number of histone modifying enzymes to the chromatin including p300 and/or CREB binding protein (CBP), both of which have histone acetyl-transferase (HAT) activity and have been shown to potentiate long term memory formation (Vecsey et al., 2007). Despite the promising role of CREB in promoting stress-induced epigenetic modifications, investigations into its activation in response to forced swim stress revealed widespread activation throughout the dentate gyrus, the rest of the hippocampus and many other brain areas (Bilang-Bleuel et al., 2002). The widespread phosphorylation of CREB in dentate gyrus neurons after psychological challenges such as forced swimming presents a conundrum which will be addressed further below.

An alternative downstream target of ERK activation is E twenty-six (ETS)-domain protein (Elk-1), a transcription factor which can bind to Elk binding sites in serum response elements of gene promoters and recruit HATs such as p300 (Li et al., 2003). Immunohistochemical studies identified pElk-1 expression in the same neurons that were positive for H3S10p-K14ac, pERK1/2 and pMSK1 confirming a potential role for this signaling molecule in acetylation of H3 (Gutierrez-Mecinas et al., 2009).

Expression of the immediate early gene *c-fos* is commonly used as a marker for neuronal activation as it is often expressed when neurons become activated (Hoffman et al., 1993). *c-*

1 Fos is a transcription factor which acts in conjunction with other proteins to induce
2 transcription of a wide variety of genes. Expression of c-Fos in the dentate gyrus was
3 increased after exposure to forced swim stress and immunohistochemical staining
4 demonstrated that c-Fos was expressed in specific H3S10p-K14ac, pERK1/2, and pMSK1
5 positive granule neurons within the dentate gyrus (Chandramohan et al., 2007; 2008;
6 Gutierrez-Mecinas et al., 2009). More recent studies have provided evidence for the
7 phospho-acetylation of histone H3 tails within the *c-fos* promoter by ChIP and real time
8 polymerase chain reaction (qPCR) analysis, finding an enhanced enrichment of H3S10p-
9 K14ac in the *c-fos* promoter after exposure to forced swim stress (Gutierrez-Mecinas et al.,
10 2009; Trollope and Reul, unpublished results). It therefore seems likely that c-Fos expression
11 is induced by phospho-acetylation of histone H3 and may be the link between biochemical
12 signaling pathway activation and changes in target gene expression.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

31 In view of the strong transactivation potential of pCREB regarding *c-fos* gene transcription
32 (Berkowitz et al., 1989; Boutillier et al., 1992; Herdegen and Leah, 1998), it is obvious that
33 the forced swimming-induced sparse induction pattern of c-Fos in dentate granule neurons in
34 the face of a virtually ubiquitous activation of CREB in these neurons is a striking
35 conundrum. Basically, the question is why is c-Fos only sparsely induced in dentate neurons
36 when pCREB is generated in all neurons? Our recent signaling and epigenetic studies may
37 provide an answer to this question. We have shown that the induction of c-Fos in dentate
38 neurons requires the activation of GRs in conjunction with the NMDA/ERK1/2/MSK1-Elk-1
39 signaling pathway which results in the phosphorylation and acetylation of histone H3 tails.
40 This combinatorial histone mark is known to be involved in the opening of condensed,
41 inactive chromatin rendering it available for transcription (Cheung et al., 2000; Clayton et al.,
42 2000; Nowak and Corces, 2000). As transcription factors like pCREB require an open
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 chromatin structure in order to transactivate gene promoters, the sparse induction pattern of c-
2 Fos in dentate neurons is most likely the result of an open chromatin configuration of this
3 gene evoked by the phospho-acetylation of histone H3 specifically in these neurons. In
4 dentate neurons in which the ERK MAPK pathway is not activated and dual histone marks do
5 not evolve, the chromatin containing the *c-fos* gene will remain condensed and inactive and
6 any generated pCREB will not be able to transactivate the *c-fos* gene promoter. Thus, it
7 seems that in dentate granule neurons GR and NMDA-ERK-MAPK driven histone
8 modifications and subsequent chromatin remodeling is a prerequisite for the transactivation
9 potential of transcription factors like CREB. In other parts of the brain c-Fos induction does
10 not require histone H3 phospho-acetylation and thus in these areas the gene seems to be in an
11 open chromatin configuration. Accordingly, in areas such as the neocortex, a widespread
12 CREB activation is matched by a widespread c-Fos induction (Bilang-Bleuel et al., 2002).
13
14 The details of our concept are outlined in Figure 2.

15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34 The hippocampus expresses high levels of GR and is responsible for co-ordination of
35 neuroendocrine and behavioral responses to stress making it a prime site for studies of the
36 stress response *in vivo* (Reul and de Kloet, 1985; De Kloet and Reul, 1987; De Kloet et al.,
37 2005). In the early 1980s it was observed that the behavioral immobility response, as
38 displayed in the forced swim re-test, is strongly dependent on glucocorticoid hormones
39 secreted during the initial forced swim challenge and acting via GRs (Veldhuis and De Kloet,
40 1983; Jefferys et al., 1983). Follow-up work demonstrated that specifically GRs in the dentate
41 gyrus are crucial for the forced swimming induced behavioral immobility response (De Kloet
42 et al., 1988). Using the selective receptor antagonists RU38486 and ORG 34517 we
43 examined whether there was a relationship between the histone H3 phospho-acetylation
44 response and the behavioral immobility response following forced swimming. The forced
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 swimming induced increases in both H3S10p-K14ac positive neurons in the dentate gyrus
2 and immobility behavior in the 24-h re-test were dependent on GR activation. Thus, in recent
3 years a picture has emerged indicating that the induction of H3S10p-K14ac and c-Fos in
4 dentate granule neurons as well as the consolidation of the behavioral immobility response
5 require signaling through both the GR as well as the NMDA-ERK-MAPK pathways (Bilang-
6 Bleuel et al., 2005; Chandramohan et al., 2007; Reul and Chandramohan, 2007; Reul et al.,
7 2009). Blockade of either pathway lead to abrogated epigenetic, gene expression and
8 behavioral responses. Recent work suggests that the interaction of signaling pathways is not
9 occurring at the genomic level but rather through direct cross talk between participating
10 signaling molecules at the protein-protein level. After forced swimming, GR activation
11 enhances the downstream activation of the ERK1/2 dependent kinases MSK1 and Elk-1
12 resulting in enhanced responses in histone H3 phospho-acetylation and c-Fos induction in
13 dentate granule neurons (Gutierrez Mecinas et al., 2009). This is a novel non-genomic
14 mechanism of GC hormone action.
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 A serendipitous finding emerging from the above studies found a decrease in
37 hyperacetylation of histone H4, another epigenetic modification, following forced swim
38 stress at multiple locations within the brain (neocortex, hippocampus etc) (Trollope and Reul,
39 unpublished results). In contrast, the stress-induced increase in H3 phospho-acetylation
40 (H3S10p-K14ac) and c-Fos was specifically located to the dentate gyrus, with the majority of
41 positive neurons situated amongst the mature neurons of the middle and outer aspects of the
42 granular cell layer in the dorsal blade of the dentate gyrus (Bilang-Bleuel et al., 2005;
43 Chandramohan et al., 2007; 2008). Since young, immature neurons of the dentate gyrus are
44 located in the inner aspect of the granular cell layer, close to the subgranular zone, this
45 finding indicates a high degree of neuroanatomical specificity with regard to this molecular
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 response to stress and highlights how the accessibility of a gene may vary between cell
2 populations and tissues depending on chromatin conformation.
3
4
5
6

7 **Epigenetics: role in learning and memory**

8
9 Adaptive and coping behaviors such as immobility in the forced swim retest and freezing in
10 the fear conditioning retest are dependent on successful memory formation during initial
11 training/tests. GR activation during the consolidation phase of the forced swim test is
12 required for the normal expression of behavioral immobility in the retest. Studies in learning
13 and memory tests such as contextual fear conditioning and Morris water maze learning have
14 identified a similar dependence on GR at a critical stage in memory consolidation hinting that
15 these tests may all share a central pathway for memory formation (Oitzl and de Kloet, 1992;
16 Cordero and Sandi, 1998; Revest et al., 2005). Histone modification and c-Fos induction have
17 also been shown to occur in response to a number of learning and memory paradigms *in vivo*
18 (Chwang et al. 2007). In these studies, ERK1/2 inhibition (by the specific MEK inhibitor
19 U0126) and MSK1 knockout mice were used in fear conditioning and Morris water maze
20 tests to identify an ERK1/2-MSK1-H3S10p-K14ac dependent signaling pathway in the
21 mouse hippocampus. This pathway was responsible for the adaptive behavior learnt and
22 displayed in these tests; blocking this pathway blocked the learned behavior (Chwang et al.,
23 2007). This ERK1/2-MSK1-H3S10p-K14ac pathway identified by Chwang et al. (2007) is
24 analogous to the one we identified in response to forced swim and novelty stress indicating
25 that a common pathway is activated by a range of stressful learning paradigms.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

53 Memory formation plays a key role in both stress responses and learning paradigms, and
54 epigenetic modifications are critical in the response to a variety of stressors such as forced
55 swim, Morris water maze and fear conditioning (Chwang et al., 2007; Chandramohan et al.,
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2008). Taken together, these observations indicate that epigenetic modifications underlying changes in gene expression may be contributing to the process of memory formation and/or retrieval alongside previously identified modulators of learning and memory processes.

Application of the signaling pathways to the chromatin during immune stress

The focus of this review has been the role of epigenetic modifications in psychological stress responses and memory formation but there is evidence in the literature of similar pathways playing a role in the epigenetic response to immune stress. This may not be surprising since the immune system is highly integrated with the neuronal and endocrine systems and interacts significantly with key modulators of both systems (Besedovsky and Rey, 2007).

GCs, released after HPA axis activation, modulate the expression of a broad range of cytokines (Wiegers and Reul, 1998). The traditional view is that GCs generally inhibit proinflammatory cytokine expression but in other studies GC release increases the expression of receptors for these proinflammatory cytokines (Wiegers and Reul, 1998; Liberman et al., 2007). This apparent conundrum may be explained if the role of GCs is to ‘optimise the course of a biological response’ and regulate the balance between pro- and anti-inflammatory cytokine expression (Wiegers and Reul, 1998; Elenkov and Chrousos, 2002). One mechanism by which GCs may optimise the biological response was proposed by Wiegers and Reul (1998). They used the example of T-cell proliferation to demonstrate that the action of GCs to reduce cytokine expression (in this case IL2) is not necessarily paralleled by a reduction in biological response. Indeed, the fact that GCs reduced the time of the peak expression of IL2 receptor (IL-2R) by two days actually resulted in a faster, superior biological response compared with the response in the absence of GCs (Wiegers et al., 1995; 2001). This GC-induced optimisation was possible because it is the receptor expression, and not cytokine

1 availability, which is the limiting factor in this phase of the response (Wiegers and Reul,
2 1998). They showed that CD4 levels were increased during GC-induced rises in T-cell
3 proliferation (Wiegers et al., 2000). Furthermore, these GC-induced responses were
4 desensitized if the rats had experienced high circulating GC levels for an extended period of
5 time (Sterzer et al., 2004).
6
7
8
9
10

11 Interleukin 4 (IL-4) is an anti-inflammatory cytokine produced by T-cells which acts in an
12 autocrine manner to elicit a key role in the differentiation of T-cells. GCs regulate the
13 expression of this cytokine indirectly by removing the inhibitory control of IL-4 by IL-12
14 (Elenkov and Chrousos, 2002). In addition to its role as an anti-inflammatory agent, IL-4 has
15 a neuromodulatory function and its receptor is functionally expressed in the granular neurons
16 of the dentate gyrus in adult rats (Nolan et al., 2005). Preventing IL-4 production by T-cells
17 in meningeal spaces severely impairs performance in behavioral studies targeting learning
18 and memory processes such as the Morris water maze (Derecki et al., 2010). Further
19 investigations found a strong correlation between IL-4 production and upregulation of brain-
20 derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus. BDNF is
21 associated with neural plasticity and is an important factor in the consolidation of memories
22 (Schaaf et al., 2000). Since the hippocampus is one of the key sites for memory processing, it
23 was hypothesised that meningeal T cell-derived IL-4 production is enhancing cognitive
24 function via BDNF expression in the hippocampus (Takei et al., 2010). The signaling
25 pathways activated by IL-4 which are responsible for the enhanced performance in learning
26 and memory tasks is unknown, however, since BDNF expression has been accompanied by
27 increased acetylation of histone H3 and H4 (Takei et al., 2010), and since IL-4 can
28 phosphorylate MAPK and induce phospho-acetylation of H3 (H3S10p-K14ac) in T-cells
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 (Kraus et al., 2010), a similar, epigenetic based mechanism to that proposed to occur in
2 response to psychological stress (Reul and Chandramohan, 2007) is promising.
3
4
5
6

7 **Role of epigenetics in psychiatric and neurological disorders**

8
9 Although epigenetic modifications are beginning to emerge as having a role in a range of
10 diseases this review will focus on just a couple, namely post traumatic stress disorder (PTSD)
11 and multiple sclerosis.
12
13
14
15
16

17 *Post traumatic stress disorder (PTSD)*

18
19 Since the 1980's, PTSD has been recognised as a pathological anxiety disorder, which can
20 develop after exposure to a traumatic event (Yehuda and Bierer, 2009). Prior to this it was
21 thought that once the threat (stressor) was no longer present the symptomatic effects of stress
22 would disappear. It seems clear now that a certain proportion of victims of trauma (rape, war
23 situations) will develop PTSD-associated symptoms over the course of time, even after the
24 stressful situation or time period has passed. Symptoms associated with PTSD include
25 hyperarousal (alertness, possibly mediated by the release of noradrenaline), avoidance of
26 stressful situations and the recall of traumatic memories (Nair and Singh Ajit, 2008).
27
28 Physiological changes including a reduction in basal cortisol levels, an increase in
29 corticotrophin releasing hormone (CRH) in cerebral spinal fluid and increased expression of
30 GR in lymphocytes have been found in PTSD patients when compared with matched controls
31 (Yamamoto et al., 2009; Yehuda and Bierer, 2009). Since all these changes are associated
32 with the HPA axis this has lead to the notion that the pathology of PTSD is caused by
33 aberrant function of the HPA axis (Yehuda and Bierer, 2009). PTSD has also been associated
34 with abnormal immune function. Patients with PTSD show high levels of circulating
35 inflammatory markers, lower natural killer cell activity and lower T-lymphocyte counts when
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 compared with matched controls (Pace and Heim, 2011). It is unclear whether the changes in
2 immune factors are a result of the aberrant HPA axis or initiate the sensitisation of the HPA
3 axis since IL1 has been shown to elicit long-lasting changes in HPA axis function and vice
4 versa (Schöbitz et al., 1994; Linthorst et al., 1994; 1995b; Labeur et al., 1995; Linthorst et al.,
5 1995a; Reul et al., 1998; Linthorst et al., 1999; Schmidt et al., 2003).

6
7
8
9
10
11
12
13
14 Administration of FG-7142, a partial indirect GABA-A receptor inverse agonist which
15 inhibits GABA function, can induce PTSD-like symptoms in humans whereas exposure to
16 GABA-A agonists like alcohol during a traumatic experience reduce the likelihood of
17 developing PTSD (Kalueff and Nutt, 1996; Evans and Lowry, 2007). These effects may be
18 linked to epigenetic modifications since studies in rats have shown that lorazepam, a
19 benzodiazepine indirect GABA-A receptor agonist, has strong anxiolytic properties and
20 inhibits phospho-acetylation of histone H3 (H3S10p-K14ac) in response to stress
21 (Papadopoulos et al., 2010). Treatment of rats with FG-7142 has the opposite effect, i.e. the
22 animals show increased anxiety-related behavior and enhanced levels of H3S10p-K14ac-
23 positive neurons in the dentate gyrus in response to a novel environment challenge (Reul and
24 Nutt, 2008; Papadopoulos et al., 2010). It is thought that traumatic early life experiences
25 increase the likelihood of developing PTSD in later life. Work of Meaney and colleagues has
26 shown that the DNA methylation status is critical in linking early life experiences with adult
27 responses to stress (Weaver et al., 2004). Demethylation of genes involved in immune
28 function, such as toll-like receptor 1 and 3 (TLR1 & TLR3), interleukin 8 (IL8), lymphotoxin
29 alpha (LTA) and killer cell lectin-like receptor subfamily G, member 1 (KLRG1) have been
30 observed to correlate strongly with the occurrence of PTSD following a traumatic event
31 (Uddin et al., 2010). Exposure to single prolonged stress (SPS, see Table 1) provoked a
32 phenotype in rats similar to PTSD and therefore these rats were used as an animal model of
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 PTSD in subsequent studies (Yamamoto et al., 2009). When SPS/PTSD rats were assessed in
2 fear conditioning tests there was increased acetylation of histone H3 and H4 in the promoter
3 regions for exon I and IV of the BDNF gene in the hippocampus when compared with fear
4 conditioned control rats (no exposure to SPS). The changes in histone acetylation induced by
5 fear conditioning training in the animal model of PTSD was associated with increased
6 freezing behavior during the subsequent retest (Takei et al., 2010). Taken together these
7 studies indicate an emerging role for epigenetic modifications in the development and
8 prevalence of PTSD.
9
10
11
12
13
14
15
16
17
18
19
20
21

22 *Multiple sclerosis*

23
24 Multiple sclerosis (MS) is a neuroimmunological disease which is characterised by
25 demyelination of neurons in the central nervous system resulting in a number of detrimental
26 symptoms including muscle weakness, cognitive impairment including deficits in memory
27 formation and retrieval, fatigue, mood disorders and many others (Heesen et al., 2007).
28
29 Studies on MS patients and the MS animal model (experimental autoimmune
30 encephalomyelitis (EAE)) have shown an involvement of the HPA axis in the vulnerability
31 for and progression of this autoimmune disease (Stefflerl et al., 1999; 2001; Heesen et al.,
32 2007). Levels of DNA methylation in the promoters of specific genes are lower in patients
33 suffering from MS compared with healthy controls. One of these genes, peptidyl
34 argininedeiminase 2, is overexpressed in MS and promotes the citrullination (conversion of
35 arginine to citrulline) which alters how the protein is folded due to an increase in
36 hydrophobicity causing a pathological phenotype (Mastronardi et al., 2007). Exploratory
37 studies have also shown a surprising role for the histone deacetylase inhibitor TSA as a
38 beneficial treatment for MS suggesting a loss of acetylation marks is also occurring during
39 disease progression (Camelo et al., 2005).
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2 **Summary**
3

4 Our studies so far have identified a unique role for two signaling pathways (i.e. the GR and
5 ERK MAPK pathways), activated after exposure to specific stressors, which initiate
6 epigenetic changes, modification of gene expression, memory formation and resulting in
7 adaptive behavioral responses. Other research has described a role for these pathways in
8 learning and memory paradigms such as the Morris water maze. Given the integration of the
9 neuronal, endocrine and immune systems we speculate that these pathways, or components of
10 them, play a role in other adaptive responses such as adaptive immunity and homeostasis.
11 Although advances have been made to improve our understanding of the process surrounding
12 the formation of long-term memories of significant (stressful) events in our lives there is still
13 much ambiguity in the field. Epigenetic mechanisms and their role in memory formation may
14 therefore be the molecular basis for the crossover between stress studies and learning
15 paradigms as well as having a central role in hormonal and immunological adaptations.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39 **Acknowledgment**
40

41 Our work described in this review article is supported by the Biotechnology and Biological
42 Sciences Research Council (BBSRC; Grant Reference Numbers BB/F000510/1 and
43 BB/G02507X/1) as well as a Medical Research Council (MRC)-funded In Vivo
44 Neuroscience Capacity Building PhD Studentships (to A.C. and E.A.S.) of the United
45 Kingdom.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

1
2
3
4 Akbarian S, Huang H-S (2009) Epigenetic regulation in human brain-focus on histone lysine
5 methylation. *Biol. Psychiatry* 65:198-203
6

7 Arthur JSC (2008) MSK activation and physiological roles. *Front. Biosci.* 13:5866-79
8

9
10 Balakrishnan L, Milavetz B (2010) Decoding the histone H4 lysine 20 methylation mark.
11 *Crit. Rev. Biochem. Mol. Biol.* 45:440-52
12

13 Bassett JR, Cairncross KD, King MG (1973) Parameters of novelty, shock predictability and
14 response contingency in corticosterone release in the rat. *Physiol. & Behav.* 10:901-7
15

16
17 Berkowitz L a, Riabowol KT, Gilman MZ (1989) Multiple sequence elements of a single
18 functional class are required for cyclic AMP responsiveness of the mouse c-fos promoter.
19 *Mol. Cell. Biol.* 9:4272-81
20

21
22 Besedovsky HO, Rey AD (2007) Physiology of psychoneuroimmunology: a personal view.
23 *Brain, Behav., Immun.* 21:34-44
24

25
26 Bilanz-Bleuel A, Rech J, De Carli S, Holsboer F, Reul JMHM (2002) Forced swimming
27 evokes a biphasic response in CREB phosphorylation in extrahypothalamic limbic and
28 neocortical brain structures in the rat. *Eur. J. Neurosci.* 15:1048-60
29

30
31 Bilanz-Bleuel A, Ulbricht S, Chandramohan Y, De Carli S, Droste SK, Reul JMHM (2005)
32 Psychological stress increases histone H3 phosphorylation in adult dentate gyrus granule
33 neurons: involvement in a glucocorticoid receptor-dependent behavioural response. *Eur. J.*
34 *Neurosci.* 22:1691-700
35

36
37 Boutillier a L, Barthel F, Roberts JL, Loeffler JP (1992) Beta-adrenergic stimulation of cFOS
38 via protein kinase A is mediated by cAMP regulatory element binding protein (CREB)-
39 dependent and tissue-specific CREB-independent mechanisms in corticotrope cells. *J. Biol.*
40 *Chem.* 267:23520-6
41

42
43 Camelo S, Iglesias AH, Hwang D, Due B, Ryu H, Smith K, Gray SG, Imitola J, Duran G,
44 Assaf B, Langley B, Khoury SJ, Stephanopoulos G, De Girolami U, Ratan RR, Ferrante RJ,
45 Dangond F (2005) Transcriptional therapy with the histone deacetylase inhibitor trichostatin
46 A ameliorates experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 164:10-21
47

48
49 Carrasco GA, Van de Kar LD (2003) Neuroendocrine pharmacology of stress. *Eur. J.*
50 *Pharmacol.* 463:235-72
51

52
53 Champagne FA (2008) Epigenetic mechanisms and the transgenerational effects of maternal
54 care. *Front. Neuroendocrinol.* 29:386-97
55

56
57 Chandramohan Y, Droste SK, Arthur JSC, Reul JMHM (2008) The forced swimming-
58 induced behavioural immobility response involves histone H3 phospho-acetylation and c-Fos
59 induction in dentate gyrus granule neurons via activation of the N-methyl-D-
60

1 aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signalling
2 pathway. *Eur. J. Neurosci.* 27:2701-13

3 Chandramohan Y, Droste SK, Reul JM (2007) Novelty stress induces phospho-
4 acetylation of histone H3 in rat dentate gyrus granule neurons through coincident signalling
5 via the N-methyl-D-aspartate receptor and the glucocorticoid receptor: relevance for c-fos
6 induction. *J. Neurochem.* 101:815-28

7
8
9 Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD (2000) Synergistic
10 coupling of histone H3 phosphorylation and acetylation in response to epidermal growth
11 factor stimulation. *Mol. Cell* 5:905-15

12
13 Chwang WB, Arthur JS, Schumacher A, Sweatt JD (2007) The nuclear kinase mitogen- and
14 stress-activated protein kinase 1 regulates hippocampal chromatin remodeling in memory
15 formation. *J. Neurosci.* 27:12732-42

16
17 Clayton AL, Rose S, Barratt MJ, Mahadevan LC (2000) Phosphoacetylation of histone H3 on
18 c-fos- and c-jun-associated nucleosomes upon gene activation. *EMBO J.* 19:3714-26

19
20 Cordero MI, Sandi C (1998) A role for brain glucocorticoid receptors in contextual fear
21 conditioning: dependence upon training intensity. *Brain Res.* 786:11-7

22
23 Cosgrove MS, Patel A (2010) Mixed lineage leukemia: a structure-function perspective of the
24 MLL1 protein. *FEBS J.* 277:1832-42

25
26 Crosio C, Heitz E, Allis CD, Borrelli E, Sassone-Corsi P (2003) Chromatin remodeling and
27 neuronal response: multiple signaling pathways induce specific histone H3 modifications and
28 early gene expression in hippocampal neurons. *J. Cell Sci.* 116:4905-14

29
30 Davis M, Astrachan DI (1978) Conditioned fear and startle magnitude: effects of different
31 footshock or backshock intensities used in training. *J. Exp. Psychol. Anim. Behav. Process.*
32 4:95-103

33
34 Dayas CV, Buller KM, Crane JW, Xu Y, Day TA (2001) Stressor categorization: acute
35 physical and psychological stressors elicit distinctive recruitment patterns in the amygdala
36 and in medullary noradrenergic cell groups. *Eur. J. Neurosci.* 14:1143-52

37
38 De Kloet ER, De Kock S, Schild V, Veldhuis HD (1988) Antiglucocorticoid RU 38486
39 attenuates retention of a behaviour and disinhibits the hypothalamic-pituitary adrenal axis at
40 different brain sites. *Neuroendocrinology* 47:109-15

41
42 De Kloet ER, Reul JM (1987) Feedback action and tonic influence of corticosteroids on
43 brain function: a concept arising from the heterogeneity of brain receptor systems.
44 *Psychoneuroendocrinology* 12:83-105

45
46 De Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: from adaptation to disease.
47 *Nat. Rev. Neurosci.* 6:463-75

48
49 De Pablo JM, Parra A, Segovia S, Guillamón A (1989) Learned immobility explains the
50 behavior of rats in the forced swimming test. *Physiol. & Behav.* 46:229-37

1 De Quervain DJ-F, Aerni A, Schelling G, Roozendaal B (2009) Glucocorticoids and the
2 regulation of memory in health and disease. *Front. Neuroendocrinol.* 30:358-70

3
4 Derecki NC, Cardani AN, Yang CH, Quinnes KM, Carihfield A, Lynch KR, Kipnis J (2010)
5 Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J. Exp. Med.*
6 207:1067-80

7
8 Drobic B, Pérez-Cadahía B, Yu J, Kung SK-P, Davie JR (2010) Promoter chromatin
9 remodeling of immediate-early genes is mediated through H3 phosphorylation at either serine
10 28 or 10 by the MSK1 multi-protein complex. *Nucleic Acids Res.* 38:3196-208

11
12
13 Elenkov IJ, Chrousos GP (2002) Stress hormones, proinflammatory and antiinflammatory
14 cytokines, and autoimmunity. *Ann. N. Y. Acad. Sci.* 966:290-303

15
16
17 Evans AK, Lowry CA (2007) Pharmacology of the beta-carboline FG-7,142, a partial inverse
18 agonist at the benzodiazepine allosteric site of the GABA A receptor: neurochemical,
19 neurophysiological, and behavioral effects. *CNS Drug Rev.* 13:475-501

20
21
22 Green AR, Vincent ND (1987) The effect of repeated electroconvulsive shock on GABA
23 synthesis and release in regions of rat brain. *Br. J. Pharmacol.* 92:19-24

24
25
26 Gutierrez-Mecinas M, Collins A, Qian X, Hesketh S, Reul JM (2009) Forced swimming
27 evoked histone H3 phospho-acetylation and c-Fos induction in dentate gyrus granule neurons
28 involves ERK1/2-mediated MSK1 and Elk-1 phosphorylation. *Society of Neuroscience*
29 *Abstracts Available at: <http://www.abstractsonline.com/Plan/SSResults.aspx> [Accessed*
30 *November 29, 2010].*

31
32
33 Hauge C, Frödin M (2006) RSK and MSK in MAP kinase signalling. *J. Cell Sci.* 119:3021-3

34
35
36 Heesen C, Gold SM, Huitinga I, Reul JM (2007) Stress and hypothalamic-pituitary-
37 adrenal axis function in experimental autoimmune encephalomyelitis and multiple sclerosis -
38 a review. *Psychoneuroendocrinology* 32:604-18

39
40
41 Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB (2008) The link between
42 childhood trauma and depression: insights from HPA axis studies in humans.
43 *Psychoneuroendocrinology* 33:693-710

44
45
46 Herdegen T, Leah JD (1998) Inducible and constitutive transcription factors in the
47 mammalian nervous system: control of gene expression by Jun, Fos and Krox, and
48 CREB/ATF proteins. *Brain Res. Brain Res. Rev.* 28:370-490

49
50
51 Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE
52 (2003) Central mechanisms of stress integration: hierarchical circuitry controlling
53 hypothalamo-pituitary-adrenocortical responsiveness. *Front. Neuroendocrinol.* 24:151-180

54
55
56 Hoffman GE, Smith MS, Verbalis JG (1993) c-Fos and related immediate early gene
57 products as markers of activity in neuroendocrine systems. *Front. Neuroendocrinol.* 14:173-
58 213

1 Hyllus D, Stein C, Schnabel K, Schiltz E, Imhof A, Dou Y, Hsieh J, Bauer U-M (2007)
2 PRMT6-mediated methylation of R2 in histone H3 antagonizes H3 K4 trimethylation. *Genes*
3 & *Dev.* 21:3369-80

4
5 Illingworth RS, Bird AP (2009) CpG islands--□ a rough guide'. *FEBS lett.* 583:1713-20

6
7 Jefferys D, Copolov D, Irby D, Funder J (1983) Behavioural effect of adrenalectomy:
8 reversal by glucocorticoids or [D-Ala²,Met⁵]enkephalinamide. *Eur. J. Pharmacol.* 92:99-103

9
10 Kalueff A, Nutt DJ (1996) Role of GABA in memory and anxiety. *Depress. Anxiety* 4:100-
11 10

12
13
14 Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, An J, Lamperti
15 ED, Koh KP, Ganetzky R, Liu XS, Aravind L, Agarwal S, Maciejewski JP, Rao A (2010)
16 Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*
17 468:839-43

18
19
20 Korte SM (2001) Corticosteroids in relation to fear, anxiety and psychopathology. *Neurosci.*
21 *Biobehav. Rev.* 25:117-42

22
23
24 Kouzarides T (2007) Chromatin modifications and their function. *Cell* 128:693-705

25
26
27 Kraus J, Lehmann L, Börner C, Höllt V (2010) Epigenetic mechanisms involved in the
28 induction of the mu opioid receptor gene in Jurkat T cells in response to interleukin-4. *Mol.*
29 *Immunol.* 48:257-63

30
31
32 Labeur MS, Arzt E, Wiegers GJ, Holsboer F, Reul JMHM (1995) Long-term
33 intracerebroventricular corticotropin-releasing hormone administration induces distinct
34 changes in rat splenocyte activation and cytokine expression. *Endocrinology* 136:2678-88

35
36
37 Li Q-J, Yang S-H, Maeda Y, Sladek FM, Sharrocks AD, Martins-Green M (2003) MAP
38 kinase phosphorylation-dependent activation of Elk-1 leads to activation of the co-activator
39 p300. *EMBO J.* 22:281-91

40
41
42 Liberman AC, Druker J, Perone MJ, Arzt E (2007) Glucocorticoids in the regulation of
43 transcription factors that control cytokine synthesis. *Cytokine Growth Factor Rev.* 18:45-56

44
45
46 Liberzon I, Krstov M, Young EA (1997) Stress-restress: effects on ACTH and fast feedback.
47 *Psychoneuroendocrinology* 22:443-53

48
49
50 Linthorst AC, Flachskamm C, Holsboer F, Reul JMHM (1994) Local administration of
51 recombinant human interleukin-1 beta in the rat hippocampus increases serotonergic
52 neurotransmission, hypothalamic-pituitary-adrenocortical axis activity, and body
53 temperature. *Endocrinology* 135:520-32

54
55
56 Linthorst AC, Flachskamm C, Holsboer F, Reul JMHM (1995)(a) Intraperitoneal
57 administration of bacterial endotoxin enhances noradrenergic neurotransmission in the rat
58 preoptic area: relationship with body temperature and hypothalamic--pituitary--adrenocortical
59 axis activity. *Eur. J. Neurosci.* 7:2418-30

1 Linthorst AC, Flachskamm C, Holsboer F, Reul JMHM (1996) Activation of serotonergic
2 and noradrenergic neurotransmission in the rat hippocampus after peripheral administration
3 of bacterial endotoxin: involvement of the cyclo-oxygenase pathway. *Neuroscience* 72:989-
4 97

5
6 Linthorst AC, Flachskamm C, Müller-Preuss P, Holsboer F, Reul JMHM (1995)(b) Effect of
7 bacterial endotoxin and interleukin-1 beta on hippocampal serotonergic neurotransmission,
8 behavioral activity, and free corticosterone levels: an in vivo microdialysis study. *J. Neurosci.*
9 15:2920-34

10
11
12 Linthorst a C, Karanth S, Barden N, Holsboer F, Reul JMHM (1999) Impaired glucocorticoid
13 receptor function evolves in aberrant physiological responses to bacterial endotoxin. *Eur. J.*
14 *Neurosci.* 11:178-86

15
16
17 Linthorst ACE, Flachskamm C, Reul JMHM (2008) Water temperature determines
18 neurochemical and behavioural responses to forced swim stress: an in vivo microdialysis and
19 biotelemetry study in rats. *Stress* 11:88-100

20
21
22 Linthorst ACE, Reul JMHM (2008) Stress and the brain: solving the puzzle using
23 microdialysis. *Pharmacol. Biochem. Behav.* 90:163-73

24
25
26 Linthorst ACE, Peñalva RG, Flachskamm C, Holsboer F, Reul JMHM (2002) Forced swim
27 stress activates rat hippocampal serotonergic neurotransmission involving a corticotropin-
28 releasing hormone receptor-dependent mechanism. *Eur. J. Neurosci.* 16:2441-52

29
30
31 Lucki I (1997) The forced swimming test as a model for core and component behavioral
32 effects of antidepressant drugs. *Behav. Pharmacol.* 8:523-32

33
34
35 Mastronardi FG, Noor A, Wood DD, Paton T, Moscarello MA (2007) Peptidyl
36 argininedeiminase 2 CpG island in multiple sclerosis white matter is hypomethylated. *J.*
37 *Neurosci. Res.* 85:2006-16

38
39
40 Mathews HL, Janusek LW (2011) Epigenetics and psychoneuroimmunology: mechanisms
41 and models. *Brain, Behav., Immun.* 25:25-39

42
43
44 McEwen BS (2000) The neurobiology of stress: from serendipity to clinical relevance. *Brain*
45 *Res.* 886:172-189

46
47
48 McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney
49 MJ (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates
50 with childhood abuse. *Nature Neurosci.* 12:342-8

51
52
53 Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with
54 hippocampal lesions. *Nature* 297:681-3

55
56
57 Nair J, Singh Ajit S (2008) The role of the glutamatergic system in posttraumatic stress
58 disorder. *CNS Spec.* 13:585-91

59
60
61 Nakayama J, Klar AJ, Grewal SI (2000) A chromodomain protein, Swi6, performs imprinting
62 functions in fission yeast during mitosis and meiosis. *Cell* 101:307-17

1 Nestler EJ, Hyman SE (2010) Animal models of neuropsychiatric disorders. *Nature Neurosci.*
2 13:1161-9

3 Ng SS, Yue WW, Oppermann U, Klose RJ (2009) Dynamic protein methylation in chromatin
4 biology. *Cell Mol. Life Sci.* 66:407-22

5
6
7 Nolan Y, Maher FO, Martin DS, Clarke RM, Brady MT, Bolton AE, Mills KHG, Lynch M a
8 (2005) Role of interleukin-4 in regulation of age-related inflammatory changes in the
9 hippocampus. *J. Biol. Chem.* 280:9354-62

10
11
12 Nowak SJ, Corces VG (2000) Phosphorylation of histone H3 correlates with transcriptionally
13 active loci. *Genes & Dev.* 14:3003-13

14
15
16 Oitzl MS, Kloet ER de (1992) Selective corticosteroid antagonists modulate specific aspects
17 of spatial orientation learning. *Behav. Neurosci* 106:62-71

18
19
20 Olton DS, Samuelson RJ (1976) Remembrance of places passed: Spatial memory in rats. *J.*
21 *Exp. Psychol. Anim. Behav. Process.* 2:97-116

22
23
24 Pace TWW, Heim CM (2011) A short review on the psychoneuroimmunology of
25 posttraumatic stress disorder: from risk factors to medical comorbidities. *Brain, Behav.,*
26 *Immun.* 25:6-13

27
28
29 Papadopoulos A, Chandramohan Y, Collins A, Droste SK, Nutt DJ, Reul JMHM (2010)
30 GABAergic control of novelty stress-responsive epigenetic and gene expression mechanisms
31 in the rat dentate gyrus. *Eur. Neuropsychopharm.*

32
33
34 Penn NW, Suwalski R, O' Riley C, Bojanowski K, Yura R (1972) The presence of 5-
35 hydroxymethylcytosine in animal deoxyribonucleic acid. *Biochem. J.* 126:781-90

36
37
38 Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to
39 antidepressant treatments. *Nature* 266:730-2

40
41
42 Rescorla RA (1973) Effect of US habituation following conditioning. *J. Comp. Physiol.*
43 *Psychol.* 82:137-43

44
45
46 Reul JMHM, Nutt DJ (2008) Glutamate and cortisol--a critical confluence in PTSD? *J.*
47 *Psychopharmacol. (London, U. K.)* 22:469-72

48
49
50 Reul JMHM, Kloet ER de (1985) Two receptor systems for corticosterone in rat brain:
51 microdistribution and differential occupation. *Endocrinology* 117:2505-11

52
53
54 Reul JMHM, Labeur MS, Wieggers GJ, Linthorst AC (1998) Altered neuroimmunoendocrine
55 communication during a condition of chronically increased brain corticotropin-releasing
56 hormone drive. *Ann. N. Y. Acad. Sci.* 840:444-55

57
58
59 Reul JMHM, Chandramohan Y (2007) Epigenetic mechanisms in stress-related memory
60 formation. *Psychoneuroendocrinology* 32 Suppl 1:S21-5

1 Reul JMHM, Hesketh SA, Collins A, Mecinas MG (2009) Epigenetic mechanisms in the
2 dentate gyrus act as a molecular switch in hippocampus-associated memory formation.
3 *Epigenetics* 4:434-9

4 Revest J-M, Di Blasi F, Kitchener P, Rougé-Pont F, Desmedt A, Turiault M, Tronche F,
5 Piazza PV (2005) The MAPK pathway and Egr-1 mediate stress-related behavioral effects of
6 glucocorticoids. *Nature Neurosci.* 8:664-72

7
8
9 Sandi C (2011) Glucocorticoids act on glutamatergic pathways to affect memory processes.
10 *Trends Neurosci.* 34:165-176

11
12
13 Schaaf MJ, De Kloet ER, Vreugdenhil E (2000) Corticosterone effects on BDNF expression
14 in the hippocampus. Implications for memory formation. *Stress* 3:201-8

15
16
17 Schmidt ED, Aguilera G, Binnekade R, Tilders FJH (2003) Single administration of
18 interleukin-1 increased corticotropin releasing hormone and corticotropin releasing hormone-
19 receptor mRNA in the hypothalamic paraventricular nucleus which paralleled long-lasting
20 (weeks) sensitization to emotional stressors. *Neuroscience* 116:275-83

21
22
23 Schreiber SL, Bernstein BE (2002) Signaling network model of chromatin. *Cell* 111:771-8

24
25
26 Schöbitz B, Reul JMHM, Holsboer F (1994) The role of the hypothalamic-pituitary-
27 adrenocortical system during inflammatory conditions. *Crit. Rev. Neurobiol.* 8:263-91

28
29 Soloaga A, Thomson S, Wiggin GR, Rampersaud N, Dyson MH, Hazzalin CA, Mahadevan
30 LC, Arthur JSC (2003) MSK2 and MSK1 mediate the mitogen- and stress-induced
31 phosphorylation of histone H3 and HMG-14. *EMBO J.* 22:2788-97

32
33
34 Stefferl A, Linington C, Holsboer F, Reul JMHM (1999) Susceptibility and resistance to
35 experimental allergic encephalomyelitis: relationship with hypothalamic-pituitary-
36 adrenocortical axis responsiveness in the rat. *Endocrinology* 140:4932-8

37
38
39 Stefferl A, Storch MK, Linington C, Stadelmann C, Lassmann H, Pohl T, Holsboer F, Tilders
40 FJ, Reul JMHM (2001) Disease progression in chronic relapsing experimental allergic
41 encephalomyelitis is associated with reduced inflammation-driven production of
42 corticosterone. *Endocrinology* 142:3616-24

43
44
45 Sterzer P, Wieggers GJ, Reul JMHM (2004) Long-term in vivo administration of
46 glucocorticoid hormones attenuates their capacity to accelerate in vitro proliferation of rat
47 splenic T cells. *Endocrinology* 145:3630-8

48
49
50 Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM,
51 Liu DR, Aravind L, Rao A (2009) Conversion of 5-methylcytosine to 5-
52 hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* 324:930-5

53
54
55 Takei S, Morinobu S, Yamamoto S, Fuchikami M, Matsumoto T, Yamawaki S (2010)
56 Enhanced hippocampal BDNF/TrkB signaling in response to fear conditioning in an animal
57 model of posttraumatic stress disorder. *J. Psychiatr. Res.* 1-9

1 Thomson S, Clayton AL, Hazzalin CA, Rose S, Barratt MJ, Mahadevan LC (1999)(a) The
2 nucleosomal response associated with immediate-early gene induction is mediated via
3 alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG-14 kinase. *EMBO J.*
4 18:4779-93

5
6 Thomson S, Mahadevan LC, Clayton AL (1999)(b) MAP kinase-mediated signalling to
7 nucleosomes and immediate-early gene induction. *Semin. Cell. Dev. Biol.* 10:205-14

8
9
10 Uddin M, Aiello AE, Wildman DE, Koenen KC, Pawelec G, Los Santos R de, Goldmann E,
11 Galea S (2010) Epigenetic and immune function profiles associated with posttraumatic stress
12 disorder. *Proc. Natl. Acad. Sci. U. S. A.* 107:9470-5

13
14 Valinluck V, Sowers LC (2007) Endogenous cytosine damage products alter the site
15 selectivity of human DNA maintenance methyltransferase DNMT1. *Cancer Res.* 67:946-50

16
17
18 Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, Cabrera SM,
19 McDonough CB, Brindle PK, Abel T, Wood MA (2007) Histone deacetylase inhibitors
20 enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation.
21 *J. Neurosci.* 27:6128-40

22
23
24 Veldhuis HD, De Kloet ER (1983) Antagonistic effects of aldosterone on corticosterone-
25 mediated changes in exploratory behavior of adrenalectomized rats. *Horm. Behav.* 17:225-32

26
27
28 Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S,
29 Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. *Nature Neurosci.*
30 7:847-54

31
32
33 West AP (1990) Neurobehavioral studies of forced swimming: the role of learning and
34 memory in the forced swim test. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 14:863-77

35
36
37 Wiegers GJ, Labeur MS, Stec IE, Klinkert WE, Holsboer F, Reul JMHM (1995)
38 Glucocorticoids accelerate anti-T cell receptor-induced T cell growth. *J. Immunol.* 155:1893-
39 902

40
41
42 Wiegers GJ, Reul JMHM (1998) Induction of cytokine receptors by glucocorticoids:
43 functional and pathological significance. *Trends Pharmacol. Sci.* 19:317-21

44
45
46 Wiegers GJ, Stec IE, Klinkert WE, Linthorst AC, Reul JMHM (2001) Bidirectional effects of
47 corticosterone on splenic T-cell activation: critical role of cell density and culture time.
48 *Neuroendocrinology* 73:139-48

49
50
51 Wiegers GJ, Stec IE, Klinkert WE, Reul JMHM (2000) Glucocorticoids regulate TCR-
52 induced elevation of CD4: functional implications. *J. Immunol.* 164:6213-20

53
54
55 Wu SC, Zhang Y (2010) Active DNA demethylation: many roads lead to Rome. *Nat. Rev.*
56 *Mol. Cell. Biol.* 11:607-20

57
58
59 Yamamoto S, Morinobu S, Takei S, Fuchikami M, Matsuki A, Yamawaki S, Liberzon I
60 (2009) Single prolonged stress: toward an animal model of posttraumatic stress disorder.
61 *Depress. Anxiety* 26:1110-7

1 Yehuda R, Bierer LM (2009) The relevance of epigenetics to PTSD: Implications for the
2 DSM-V. *J. Trauma. Stress.* 22:427-434

3
4 Yuen P, Tsitiridis A, Kam F, Jackman J, James D, Richardson M, Williams L, Oxford W,
5 Piper J, Thomas F, Lightman S (2009) Emotional & physical stress detection and
6 classification using thermal imaging technique. 3rd International Conference on Imaging for
7 Crime Detection and Prevention (ICDP 2009):P13-P13 Available at:
8 <http://link.aip.org/link/IEESEM/v2009/i2/pP13/s1&Agg=doi> [Accessed March 8, 2011].
9

10
11 Zhang H, Zhang X, Clark E, Mulcahey M, Huang S, Shi YG (2010) TET1 is a DNA-binding
12 protein that modulates DNA methylation and gene transcription via hydroxylation of 5-
13 methylcytosine. *Cell Res.* 20:1390-3
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Legend to Figure 1.

Scheme representing the role of the epigenome in physiological and behavioral responses. Cells communicate by a number of chemical messengers which act at different locations to elicit a biological response. Generally, signaling systems exist whereby extracellular primary messengers (growth factors, cytokines, hormones etc) act via receptors at the cell surface or are transported or diffuse across the plasma membrane to induce activation of secondary messengers and/or transcription factors within the cytoplasm. Activated secondary messengers can have biochemical effects in the cytoplasm and/or are translocated into the nucleus to act on nuclear transcription factors or influence the epigenome or genome directly. The epigenome is regarded as the complex of changes, occurring at the chromatin level, which alter the expression of genes without changing the coding sequence of the genome. Changing the level of gene products, as a result of activation of signaling cascades, influences cell function and affects both the physiology and the behavior of the organism.

Legend to Figure 2.

1
2
3
4
5 **Concept on the requirement of orchestrated signaling and epigenetic mechanisms in the**
6
7 **induction of c-Fos in dentate granule neurons after a psychological challenge.** A. Under
8
9 baseline conditions the *c-fos* gene in an inactive state possibly involving methylation of the
10
11 gene promoter DNA. B. Psychological stress causes the secretion of glucocorticoid hormone
12
13 which act via GRs and the release of glutamate which acts on NMDARs to cause a rise in
14
15 intracellular Ca²⁺ levels in sparsely distributed dentate granule neurons. Ca²⁺ influx activates
16
17 CAMKII and the ERK MAPK cascade. We recently discovered that GRs seem to act like
18
19 scaffolds facilitating the activation (i.e. phosphorylation) of MSK1 and Elk-1 by
20
21 phosphorylated ERK1/2 (pERK1/2; Gutierrez-Mecinas et al., submitted). pMSK1
22
23 phosphorylates S10 residues in histone H3 tails which evokes the opening of the chromatin.
24
25 If the *c-fos* gene promoter is methylated de-methylation (by DNA de-methylases (DDMs))
26
27 will take place at this stage or earlier. CAMKII and MSK1 and possibly other kinases (e.g.
28
29 protein kinase A (PKA)) will phosphorylate CREB. pElk-1 will bind to the Elk-1 binding site
30
31 in the serum response element (SRE) and recruit and phosphorylate the HAT p300. pCREB
32
33 and GRs may also recruit HATs. The recruited HATs will acetylate the histone tails
34
35 (including H3S10p rendering it into H3S10p-K14ac) and thereby stabilize the open
36
37 chromatin configuration. This may also involve H3K4 methylation by HMTs but their
38
39 activation/recruitment mechanisms are still unknown. Dimerized pCREB can now access the
40
41 CRE within the *c-fos* promoter and transactivate gene transcription. The c-Fos response is a
42
43 transient response as, over time, glucocorticoid hormones and glutamate diminish, Ca²⁺ levels
44
45 plummet, protein phosphatase and HDAC activities rise and ultimately the *c-fos* gene will be
46
47 inactivated, possibly involving DNA methylation of the gene promoter.
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Table 1. Behavioral tests commonly used to investigate responses to stress or learning and memory paradigms.

Behavioral test	General conditions	Adaptive behavior	Reward (if applicable)	References
Novel environment	Subjects are transferred to a new environment (usually for 30 min) and their behavior recorded.	Switch from exploratory to normal behavior	Identifying there is no threat	(Bassett et al., 1973)
Radial maze	8-arm maze which tests subjects memory by forcing them to make decisions about which arm to explore.	Finding food source	Food	(Olton and Samuelson, 1976)
Morris water maze	Subjects are trained (successive trials over consecutive days) to find an underwater platform and then the platform is removed to test their memory of its location.	Finding the platform	Escape from water	(Morris et al., 1982)
Forced swim test	Subjects are placed in are large beaker of water from which they cannot touch the bottom or escape and behavior monitored	Floating	Conservation of energy	(Porsolt et al., 1977 _a ; De Pablo et al., 1989; West, 1990; Korte,

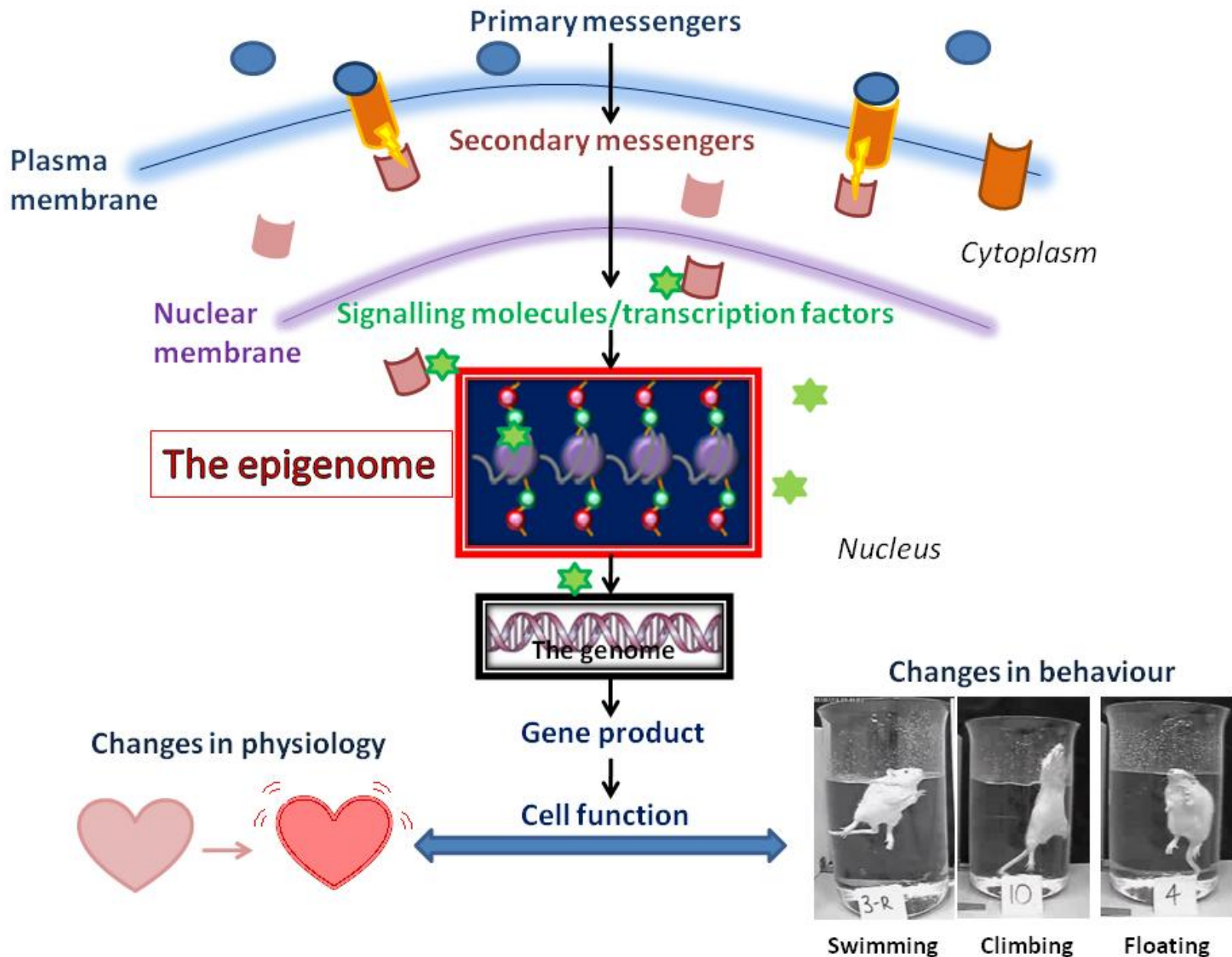
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

	<p>(usually for 15 min). A second test, usually lasting 5 min is performed after a sufficient recovery period (usually 24 h) and again behavior is monitored.</p>			<p>2001; Bilang-Bleuel et al., 2005; Chandramohan et al., 2008; Nestler and Hyman, 2010)</p>
<p>Fear conditioning</p>	<p>Subjects are placed in a box and subjected to a short-lasting foot shock, sometimes preceded by a noise or light signal (conditioned cue). Trials are often repeated before allowing the subject time to recover (varies from hours to weeks). In the retest the subject is placed in a different environment and subjected to the cue but no shock (to test cue-dependent memory) or placed in the same box with no cue or shock (to test context-related</p>	<p>Freezing</p>	<p>No reward</p>	<p>(Rescorla, 1973; Davis and Astrachan, 1978)</p>

	memory)			
Single prolonged stress	Subjects are exposed to 2 h restraint stress, followed immediately by forced swim test (usually 20 min), allowed to recover for a short period (~15 min) and then exposed to anaesthetic until unconsciousness is achieved.	Freezing	No reward	(Liberzon et al., 1997)
Immune/inflammatory stress	Can be experimentally induced by administration of bacterial endotoxins.	Sickness behavior	No reward	(Linthorst et al., 1995b)

^a Please note, this interpretation of the immobility response during forced swim retest differs from our own.

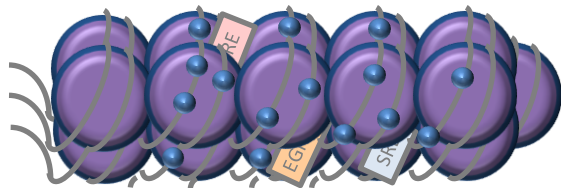
Figure(s)



Figure(s)

A. Baseline conditions

c-fos promoter
Condensed (inactive) chromatin



← DNMTs ?
→ DDMs ?

B. After psychological challenge

